contamination. But most cold-pack cheeses won't support the growth of Listeria to high levels. And even though there may have been recalls of those products, it really speaks to whether, indeed, those products represent a present, imminent danger to public health.

So, I think it is very critical that we have some kind of measure on whether these products can support growth. And I think Bruce's point is well-taken. I think there are industry folks that can help out in that assessment.

And Bob Buchanan talked about the role of challenge studies, inoculated pack studies. And there's plenty of those as well that can be factored in on that particular point.

So, to me, it goes just beyond probability of contamination, you know, whether it's contaminated there or not. But it's also level of contamination at the time of consumption, I think. I know that's a difficult thing to model.

The other thing, the other piece I guess I would ask about is the quantitative data. I know the UK data that Dr. Hitchins presented had as a upper limit greater than a thousand per gram, I believe. And given the scientific nature of the risk assessment that we're trying to do, given the work at the University of Georgia and the Emory Primate Center on the L.M. Monkey Study, as I call it, trying to get at infective dose, my question is that we try and measure up the levels of Listeria in these products that we're consuming versus anything that would come out at infective dose study, is greater than a thousand per gram or a thousand per gram sufficient enough? Or should we be trying to go higher in terms of quantitating levels?

And then, I guess my other question is the issue of, really, how do we harmonize ready-to-eat foods, definitions of ready-to-eat foods in this whole process versus, say, frozen foods, for example? And how do we factor that in as well? Thank you.

MR. MORRIS POTTER: Thanks, Paul. Other comments from -- Okay. Tony?

DR. TONY HITCHINS: Tony Hitchins, FDA. Just a comment on Paul's comments. There are data in the collection already that have, you know, numbers greater than a thousand per gram. It's just that in that particular study or that piece of that study, it wasn't apparent; it wasn't done.

MR. MORRIS POTTER: You got a little far from the mike there, Tony.

DR. TONY HITCHINS: Sorry. There is data in the data base, at least from some other studies, that gives numbers for rare cases where the counts are greater than a thousand per gram. And not all studies have that, but some do. Yeah.

MR. MORRIS POTTER: Wally?

MR. WALLY SCHLECH: Wally Schlech again. I just wanted to comment about the quantitation. I think that you also, particularly if you're looking at levels of quantitation, need to look again at the host. There is clear data in the Boston outbreak in the late 70's that antacids were a risk factor. So, you may decide to, say, allow ten to the two Listeria per gram to get out into the market. But that may not be sufficient to protect one of these immunocompromised individuals. And if you look at all the Pepcid AC ads on TV, it seems like the entire American population is swallowing them. Then maybe that would argue against -- and presumably the monkey studies might give some additional information. But we certainly have studies in a gastric model in rats that is a real phenomenon.

MR. MORRIS POTTER: Thanks, Wally. The BRFSS surveys have looked at antacid and H2 blocker consumption, at least in some of them, so there are some data there. But that may be a bit difficult to model.

MS. CARY FRYE: Cary Frye, International Dairy Foods Association. Also, the National Cheese Institute. And we really appreciate the comments here today, and we're very supportive of the risk assessment. I did speak to Mary Bender about some of the data she presented with the food consumption, specifically in the cheese category. And the slide that you showed about mandatory pasteurization of 33 percent is certainly accurate. I don't disagree with that.

However, I think commercial practices of cheese manufacturing, specifically cheese manufacturing that could have a higher probability of contamination, are showing that pasteurized milk is used. I know commercially, Mexican-style cheese by one of our members, all of their milk is pasteurized. So, it appears there could be a data gap here that might need additional information that we could assist with, rather than just looking at the regulations, but maybe providing actual practices for cheese manufacture. So, I realize that, and we hope that we can provide that because many soft cheeses are made with pasteurized milk for that very reason.

Secondly, I had a question related to the risk assessment similar to this same line of thinking. If you look at the literature, you're looking at it worldwide, cheeses that may show levels of Listeria that were made from raw milk because there's different regulations in different countries. And how will you account for that in the risk assessment? Will there be any accounting for the different practices of how cheeses are produced? Because it's my understanding the risk assessment will be looking at the risk of the U.S. population. Will you look at the imported cheeses such as the data we have at NCI and weight that, or will you look at all cheeses? Thank you.

DR. MARY BENDER: Mary Bender, FDA. There's somebody back there right now who's trying to get data, as you're discussing. Our Regulatory Affairs Office at FDA does collect some data on imports. And they're really excited that they have a data base going, but they've warned us not to take everything as is because this is a developing data base. But we have been able to look at some of the imports of the lots of cheeses. And a certain proportion that's been tested or held back for Listeria, and then some where there have been positive results. But it's been a challenge to try to put this all together to come out with something that makes sense and is accurate. There was one slide that I had that -- we do want to look at this further to try to figure out. And I really do appreciate any help.

Now, Cary and two others did come to me at the break and said that there really has not been an outbreak related to ice cream. And I looked back at my file, and there was an epidemiological link -- I don't know -- it was from a CDC article. And you all are the experts. This is something I've read. So, I really appreciate the input. Thanks.

MR. MORRIS POTTER: Other comments? Yes.

MR. LARRY BORCHERT: Larry Borchert with the American Meat Institute. My comments also deal with data acquisition and consideration. And it really is following up on points that have already been made. And I'll use that as an example. A hot dog is not a hot dog is not a hot dog as a cheese is. If we are considering international data, for example, the hot dogs that are made in Germany, for example, have probably twice the brine concentration, traces of salt and water concentration, that they do in this country. So, it warrants us to be very careful of the use of international data.

Likewise, acquisition of data, I think we do need to be cognizant of sales data. For example, two major companies in the United States produce 40 percent of the hot dogs in the United States. So, looking at broad-based consumption data might distort the overall picture, particularly if one or both of these companies are using some intervention technique that might decrease the prevalence of Listeria in their products.

So, I think the point I'm trying to make is that we must be very, very careful in acquiring the data and using the data that we are applying that to the specific products that we're talking about, not just a generic family of those particular products. Thank you.

MR. MORRIS POTTER: Thanks, Larry. Other comments? Seeing none, the schedule calls for us to be back in session at 1:00. Since we're a little ahead of schedule, I hope folks will be prompt. We will start again at 1:00. Be here.

(Whereupon, a lunch recess was had in this matter.)

MR. MICHAEL JAHNCKE: Welcome back, everybody. I hope everyone had a nice lunch. We're going to get started. We have two more presentations this afternoon-- three more, with the summary. I'm just waiting for a slide. Here we go.

As I mentioned, we're going to have two more presentations. Then Dr. Whiting later will do a summary of what has been presented to this day. We're in the session of Hazard Assessment. And the two presenters will be Dr. Pat McCarthy looking at some epidemiologic records. And the second speaker will be Dr. Richard Raybourne on dose-response experimentation.

Let me introduce our first speaker, Dr. Pat McCarthy. And he will be speaking on epidemiology of Listeria monocytogenes outbreaks.

DR. PATRICK McCARTHY: Good afternoon. I'm going to talk about the epidemiology of Listeriosis.

Next slide, please. Listeria was first described in 1926. And a few years later, the organism was recognized as a human pathogen. The suggestion that Listeria, Listeriosis could be transmitted to humans in food dates back to the 1930's. But it was not until the 1980's that evidence was obtained that Listeriosis is a foodborne disease.

Since the 1980's, foodborne outbreaks in sporadic cases have been reported in many countries throughout the world. And in 1986, the Council of State and Territorial Epidemiologists recommended that Listeriosis be a reportable disease.

Next slide. Listeria is the name of a group of disorders caused by Listeria. Listeriosis is the name of a group of disorders caused by the organism, Listeria monocytogenes. Listeriosis is clinically defined when Listeria is isolated from blood cultures, spinal fluid or an otherwise normally-sterile site like a placenta or a fetus.

Cases of Listeriosis are usually divided into perinatal and nonperinatal groups. The perinatal group includes pregnant women and their fetus or newborn. Women may get Listeriosis at any time during pregnancy, but most cases are reported in the third trimester.

Often, pregnant women will present with an influenza-like illness which includes fever, chills and headache. This prodromal illness occurs in about two-thirds of women with pregnancy-associated Listeriosis. About three to seven days after the onset of prodromal symptoms, women will abort the fetus or will have premature labor.

In the first trimester, Listeriosis results in spontaneous abortions. In later stages of pregnancy, the result can be a stillbirth or a critically-ill newborn. Sepsis occurs in about 30 percent of pregnant women with Listeriosis, and there are a few reports of meningitis in pregnant women. The fetus can suffer abortion, stillbirth. And the newborn can present with sepsis, meningitis or can die.

The nonperinatal group includes all non-pregnant persons over the age of 28 days. Nonperinatal cases primarily include persons that are taking immunosuppressive medications, persons with chronic debilitating diseases like cancer, diabetes or alcoholism, and persons over the age of 60. Healthy children and adults have a relatively low risk of infection from Listeria.

When infection does occur in children and adults, Listeriosis is usually superimposed upon some other illness. Nonperinatal cases often present with meningitis or sepsis.

In the next few minutes, I'll discuss the early foodborne outbreaks and surveillance for Listeriosis; and I'll provide some examples of recent outbreaks and sporadic reports.

Listeriosis is known to cause severe illness, but there have been events in which the majority of cases developed mild symptoms. I'll identify a few events where mild symptoms were primarily reported.

I have a slide on the incubation period for

Listeriosis and another slide on fecal carriage studies. I'll show you the incident trend for Listeriosis in the United States between 1989 and 1993. And I have some recent data from FoodNet, the ongoing active surveillance program for foodborne diseases.

Next slide. The earliest evidence that Listeriosis is a foodborne illness was obtained from outbreaks that occurred in Nova Scotia, Massachusetts, Los Angeles, and Switzerland between 1981 and 1987. Other outbreaks occurred before 1981, but the vehicle of infection was not identified. These outbreaks during the 80's lasted for several months each but involved relatively few cases. On the other hand, there were several deaths associated with these outbreaks.

Next slide. Both nonperinatal and perinatal cases were identified in each outbreak. The age range for the nonperinatal cases was between age 21 and 100. The median age in the nonperinatal cases was about 60 years. In these outbreaks, the majority of the nonperinatal cases were taking immunosuppressive medications, had a debilitating disease or were over age 60. About one-third of the nonperinatal cases died.

In the perinatal group, the mother and fetus or newborn was considered as a single case. The fatality rate in the perinatal group was about one-third.

Matched case-control studies implicated a particular food in each outbreak. In Nova Scotia, coleslaw was implicated. And dairy products were implicated in the other outbreaks. The odds ratios that implicated the food were all significant at the 0.05 level or below the 0.05 level. Listeria monocytogenes 4b was isolated from cases in each of the outbreaks and from the implicated food in all outbreaks except from Massachusetts.

The incident rates that I show here are for the populations in which the outbreaks occurred. I don't have the background incident rates for all these outbreaks. But in Switzerland in the years preceding the outbreak, the background rate was approximately .5 per hundred thousand cases. At the end of the outbreak, the incident rate was about 5 cases per 100,000. Low-incident rates make the outbreaks very difficult to detect. These outbreaks were only detected because all the cases occurred in a single hospital or were reported to a single laboratory. For example, in Los Angeles, a hospital infectious control nurse noticed the increase in cases; and her observation led to the investigation which implicated the Mexican-style cheese.

The likely source of Listeria in the Nova Scotia outbreak was the raw manure used to fertilize the cabbage which was made into coleslaw. The sources for all these outbreaks suggest that Listeriosis was linked to the farm or to food production facilities.

These early outbreaks showed that Listeriosis, the foodborne Listeriosis can cause abortion, stillbirth, sepsis, meningitis and death. Matched case-control investigations showed that significantly more cases than controls ate the implicated food. The L. monocytogenes 4b was identified in most of the infections occurring during the epidemic period. And the epidemic strain of Listeria monocytogenes was isolated from opened and unopened samples of food implicated in 3 of the 4 outbreaks.

Following the Los Angeles outbreak in 1985, CDC started Listeria surveillance. I show here data from two surveillance populations, but there were other reports in the literature of surveillance that took place between 1985 and 1993. There were 34 million people in the 1986 surveillance. And between 1989 and 1993, in that surveillance, there was 19 million people. Both surveillance periods included people from Oklahoma, Tennessee and Los Angeles County. The 1986 surveillance population was larger because health departments in Missouri, New Jersey and Washington were included.

Before the surveillance was started, hospitals, laboratories and physicians in the surveillance area were contacted and asked to report cases of Listeriosis. At the end of the surveillance period, facilities that reported cases were audited to determine the sensitivity of the surveillance. The case ascertainment for the 1986 surveillance was 93 percent, and case ascertainment in 1993 was shown to be 97 percent.

246 cases were reported in 1986. And between 1989 and 1993, about 400 cases were reported. Now, I'm going to show additional data from the 1986 surveillance. And in a few minutes, I'm going to show the incident trend that was developed for Listeriosis between 1989 and 1993.

Overall, in 1986 there were .7 culture positive cases of Listeriosis per 100,000 population. The rate was slightly less in the nonperinatal group but was much higher, 7.8, in the perinatal group.

If Los Angeles County was included, the cases per 100,000 would be approximately 24. But Los Angeles County experienced an outbreak during 1985, and this heightened awareness could have been the reason for the increase in cases. So, I have excluded it in what I'm reporting to you.

Listeria monocytogenes has 13 serobars. But 3 serotypes accounted for approximately 96 percent of the cases. 1/2a accounted for 30 percent; 1/2b for 33 percent; and 4b accounted for 33 percent of the isolates during the 1986 surveillance. Based on surveillance data, it was projected that about 1700 cases and 450 deaths due to Listeriosis occurred in the United States in 1986.

Next slide. Listeria monocytogenes can cause illness if it penetrates the lining of the GI tract. Once the organism penetrates the tissue, it can protect itself from phagocytosis, grow and then migrate throughout the host. The chance of tissue invasion is thought to depend upon the number of organisms consumed, host susceptibility and virulence of the organism.

In the 1986 surveillance, there were 179 nonperinatal cases. There was a 2-month-old and a 3-year-old, but the other 177 cases were all age 16 or over. 56 percent of the cases occurred in males; 66 percent of the cases had sepsis; 19 had sepsis and meningitis; and 12 percent had meningitis only. About 3 percent of the cases had a focal infection caused by

Listeria. The incidence of Listeriosis increased with age. 84 percent of the cases were over age 50, and 40 percent of the cases were over age 70. In adults, fatalities also increased with age. Overall, there was a 35 percent fatality rate. In cases over age 60, the fatality rate was 41 percent.

There were 67 affected pregnancies. 80 percent of the pregnancies resulted in live birth, and one of the neonates died. Of the live births, 75 percent were culture positive, so transmission of Listeria to the fetus does not always occur. 80 percent of the culture positive babies had an early onset Listeriosis.

Early onset is defined as a case of Listeriosis in a neonate between birth and seven days of age. Early onset is often characterized by a premature birth, respiratory distress and circulatory failure. In 1986, 80 percent of the early onset neonates had sepsis, and 20 percent had meningitis.

20 percent of the culture positive babies had late onset Listeriosis. Late onset is defined as Listeriosis in a neonate between 8 days and 28 days of life. Usually late onset neonates are born healthy and at fullterm. Meningitis is more common in the late onset babies. The mothers of late onset babies usually had an

unaffected pregnancy and no prodromal illness. Listeria is rarely isolated from the mother, and the source of Listeriosis is often not identified in late onset cases.

Data was available for 31 maternal cases in the 1986 surveillance. 58 percent of the mothers experienced premature labor or premature membrane rupture; 32 percent of the mothers had sepsis or fever; and 10 percent aborted their fetus. There was no meningitis and no deaths reported in the maternal cases. Listeriosis is rarely life-threatening to the mother. Other studies in the literature suggest that Listeria does not cause repeated abortions in the same women.

Next slide. This slide shows a few examples of outbreaks and sporadic reports of Listeriosis that have occurred since 1988. Listeriosis has been reported in several countries, and a variety of foods have been implicated as the vehicle of infection, including turkey franks, cheese, mushrooms, pate, fish and hot dogs.

This slide shows some of the milder symptoms that have been associated with Listeria infection. It's been estimated that 33 percent of all cases give mild symptoms and that most cases occur sporadically. Mild symptoms include chills, diarrhea, nausea, vomiting, fatigue, abdominal cramps. Reports of mild symptoms suggest the possibility that many illnesses caused by Listeria may go unreported.

This slide shows events where most of the cases reported mild symptoms -- not all the cases, but most of the cases. Again, mild symptoms associated with Listeria infection have been reported in several countries, and a variety of foods have been implicated as the vehicle of infection.

I'd just like to speak a little bit about the cases in Denmark. These cases involved babies at a daycare center. There was a 2-year-old that got fever and was hospitalized. After the fever subsided, he got diarrhea. Blood and stool cultures were obtained. The child was treated for his symptoms and released after two days in good clinical condition. After discharge, blood cultures grew Listeria monocytogenes. The baby was readmitted but no longer had symptoms. Two other babies that attended the same daycare were also admitted to the hospital, released in good condition and then readmitted when the blood culture came back positive. After the second admission, blood cultures from all three babies were negative, but stool cultures grew Listeria monocytogenes 4b. The source of the outbreak was not established. But this example shows that mild symptoms can occur even if a blood culture is positive.

The peer reviewed literature shows that the incubation period associated with Listeria infection can range from less than 24 hours to approximately 3 months. Incubation associated with severe illness, like sepsis and meningitis, can range between several days to a few months. The incubation period associated with gastrointestinal symptoms can range between several hours and a few days.

The large bowel is the principal reservoir for Listeria in humans. Several studies have looked at fecal carriage to gain insight into how the disease is transmitted, especially in sporadic cases. I show here two examples of fecal carriage studies.

In Germany, less than 1 percent of persons with diarrhea and healthy food workers were fecal carriers. In Scotland, approximately 2 percent of pregnant women and 3 percent of nonpregnant women were fecal carriers. In the literature, estimates of fecal carriage ranges between less than 1 percent to 21 percent.

It's not known how fecal carriage relates to the length of incubation or to the occurrence of Listeriosis, although it's been suggested that in fecal carriers, stress can undermine resistance; and then carriers can get the disease.

This is the Listeriosis incident trend from the 1989 to 1993 surveillance. The bar chart shows cases per million on the y-axis and year on the x-axis. About 1990, as more information became available, the regulatory agencies and private industry developed plans to reduce the incidence of Listeriosis.

Industry initiated HACCP programs and increased sanitation to eliminate contamination. The regulatory agencies expanded programs to remove contaminated foods before retail sale. There was also a consumer education campaign that focused on food safety.

Shortly after these efforts were initiated, Listeriosis declined from about 7.9 cases per million in 1989 to about 4.4 cases per million in 1993. The decline occurred in diverse geographic areas of the United States. And also, about the same time, Listeriosis declined in the United Kingdom after the government issued a health warning.

This data is from FoodNet. FoodNet is an active surveillance program. The purpose of FoodNet is to determine the frequency and severity of foodborne illness. To identify all cases of confirmed disease, FoodNet personnel contact each clinical laboratory in each surveillance area in each catchment area, either weekly or monthly.

This slide shows Listeriosis compared to other pathogens that are tracked by FoodNet. There were approximately .5 cases per 100,000 population in 1998. Data for 1996 and 1997 also showed that there was approximately .5 cases per 100,000 population in those years.

This chart shows FoodNet data from 1997. The y-axis shows cases per 100,000, and the x-axis shows ages in years. From this graph, you can see that most cases occur in the very young and in the very old. When this same data was broken down by sex, the ratio of males to females was approximately equal. This is approximately the same picture that you would see from the 1986 surveillance.

A seasonal trend of Listeriosis has been referred to in literature for many years. This slide shows combined FoodNet data from 1986 and 1997. The y-axis shows cases per month per million population. And the x-axis shows month of the year. There's an apparent increase in cases between late spring to autumn, but the reason for this apparent increase is not known.

This graphic shows some of the pathogens that are being tracked by FoodNet on the y-axis. On the x-axis, it shows the percent of isolates from hospitalized individuals. Listeria had the highest hospitalization rate in 1998. Compared with other pathogens like Salmonella and Shigella, which occurred more often, Listeria put more people into the hospital on a percent basis.

Listeriosis also had the highest hospitalization rate and the highest case fatality rate in 1997, 1998.

In conclusion, I found by reviewing the literature that Listeriosis is a deadly foodborne illness that can be transmitted in many foods, but it is not product specific. Of the FoodNet pathogens, Listeria has the highest hospitalization rate and the highest case fatality rate. Listeriosis cases could possibly increase in the future due to our aging population and to the use of immunosuppressive medications in surgery and due to the AIDS epidemic. And intervention may decrease cases of Listeriosis in the future. That's the end of my presentation.

MR. MICHAEL JAHNCKE: Thank you, Dr. McCarthy. Are there questions from the subcommittee? Bruce?

MR. BRUCE TOMPKIN: This is Bruce Tompkin. On the conclusion, it states that Listeriosis is not product specific. And in a general sense that may be true; however, it is product-specific in terms of those foods in which multiplication can occur.

DR. PATRICK McCARTHY: What I tried to point out there is that it's in hot dogs; it's in vegetables; it's in a variety of foods. And in that sense, it's not product-specific.

MR. BRUCE TOMPKIN: So, within each of those commodities, it is product-specific is what I was saying.

MR. MICHAEL JAHNCKE: Thank you. Other questions? Yes, Mike.

MR. MICHAEL DOYLE: This is Mike Doyle. Could you elaborate on this outbreak in Finland that was associated with butter?

DR. PATRICK McCARTHY: I don't think I'm prepared to at this time. I'd need some more time before I could talk about that.

MR. MICHAEL JAHNCKE: Other questions? Yes.

MR. MORRIS POTTER: Morris Potter. I'd just like to point out for the committee that three of the areas covered by surveillance in the last case-control study fall into the FoodNet catchment area, so while all of the studies on Listeriosis aren't the same, there is some overlap that allows one to look for general trends.

MR. MICHAEL JAHNCKE: Thank you. Any other questions?

Thank you very much for an excellent presentation. Thank you.

DR. PATRICK McCARTHY: Thank you.

MR. MICHAEL JAHNCKE: Our next speaker is Dr. Richard Raybourne. He will be addressing characteristics of Listeria monocytogenes, dose-response.

DR. RICHARD RAYBOURNE: I'd like to thank the committee for the opportunity to make this presentation and also to thank the collaborators in the dose-response effort whose names are listed there and two of whom are in attendance today.

Next slide, please. There are probably many ways to define -- or at least several ways to define dose-response and the concept of the dose-response model. I've chosen one that was in one of the other Listeria risk assessments by Farber, et al., and that is the dose-response model provides a functional relationship between the probability that an individual will contract Listeriosis and a specific dose or level of exposure to a virulent strain of Listeria monocytogenes. And I thought that was a reasonable definition, and I didn't think I could improve on it very much. So, I just lifted it from the paper.

In looking at the possible sources for information on dose-response, there are four listed here. The first we've heard something about in Dr. McCarthy's previous talk -- that is, the epidemiology and case report information. In addition to that, other possible sources include animal studies and in-vitro studies of various sorts which have addressed questions which are also related to dose-response.

Go on to the next slide, please. Some of the parameters that might go into calculating or developing a dose-response model are, obviously, the number of organisms; the food matrix or the food in which the organisms are existing at the time that they are consumed; the virulence of the particular Listeria strain; and the host susceptibility -- that is, the resistance or susceptibility of the host to infection.

By combining these various factors, you would develop several types of outcomes ranging all the way from asymptomatic carriage of Listeria through more mild diarrheal-type illness to invasive disease to the ultimate end point of death in some individuals and also the fetal abortions, as well.

The first issue I'm going to touch on is the issue of the food matrix. And this goes to the point that was made earlier in regard to the data initially on survival of Listeria in various foods, except the way that I'm presenting or thinking of it here is in the more qualitative sense of the effects of the types of treatment as opposed to the quantitative or number of things -- that is, to raise the question of whether adaptation of Listeria to a acidic or a high-salt environment can actually alter or result in the selection or adaptation of a functionally more virulent population of Listeria such as improving its ability to survive the stomach acid barrier or within some host phagocytic cells, as well as a result of adaptation to a harsh environment. Whether the specific environment, the specific stress in the food environment is actually the same stress may not actually be relevant due to the sort of global stress responses in some of these organisms resulting in the phenomenon that's sometimes referred to as cross tolerance among these pathogens.

In addition, another area that might well be considered is the issue of the fat content in foods, specifically again the question of whether a high-fat content and the sort of relationship between Listeria and the structure of the food and the fat mice cells, for example, could actually protect Listeria from gastric acid or even modulate its interaction with some host cells, perhaps.

I have not directly found a tremendous amount of evidence on this area. But I did find one reference in -- I think it was in the Massachusetts outbreak where there was actually a protective effect of skim milk versus whole or 2 percent milk on one outbreak. I think this is an area where additional data would also be needed.

Moving on from the food matrix issue to the area of numbers of organisms associated with illness, this is a collection of basically case report and epidemiological data which contains some dose information in it in which an effort was made to quantify the level of Listeria. And in some cases, an effort was also made to determine what the consumption was to actually get to a dose. So, in these cases where it just says, "The dose was a given CFU," that means that it was normalized for food intake. And in those where it says, "CFU per gram," it means that the intake of the food was uncertain. So, we don't actually know how much was consumed.

Again, there may be other cases that I don't know about or that our group doesn't know about. And we would definitely appreciate information related to dose from any other sources that the audience may know of.

What you can say about this is that there's certainly a wide range of doses, and they're basically all over the place in terms of the level of Listeria implicated in illness. This type of data and various other subsets of data like this have been used in three other Listeria risk assessments to produce dose-response models.

The next slide, please. In the dose-response of studies in the Farber, et al. risk assessment, they developed the dose-response curves for both high -- normal populations and high-risk populations based on a Weibull-Gamma model. In this particular graph, it plots the total number of Listeria monocytogenes cells versus the probability of illness. This was based on approximate ID-10 and ID-90 doses which were extrapolated from case report information.

In another risk assessment, Buchanan, et al. developed a conservative model using consumption data for a single food source and Listeria incidence data. In this dose curve, the plot is again the log of Listeria monocytogenes cells versus the probability of illness.

Finally, more recently, another risk assessment was done for Listeriosis derived from soft cheese consumption. Again, this used the same mathematical model. This is a little bit harder to sort of access what the cystograms represent. But I will explain that the plot here, the risk of illness from one serving of cheese versus the probability of illness. The upper curve represents the curve for the high-risk population, and the lower curve represents the low-risk population.

The point here is not to particularly dwell on these models but to make the point that there are some limitations to the approach used in these studies. And, clearly, these are all based on epidemiologic data which -- in addition to this, in these studies, the virulence is basically assumed in the sense that virulence would be considered a more or less absolute characteristic, either virulent or avirulent, and that the host susceptibility in both of these studies -- in all three of these risk assessments -- was identified as an important variable. However, in terms of developing ways to address the issue of relative susceptibility, this was essentially based on, to use the term quoted from one of the studies, a "rough approximation of the relative susceptibility."

So, for the rest of the time, I'm going to try to present some approaches by which we could use some other data sources other than the epi-data and case report data to try to improve the level of -- or decrease the level of uncertainty in these dose-response models, particularly dwelling on the issues of pathogen virulence and host susceptibility.

And so, I'm going to present some animal and various other kinds of -- and other kinds of data, in-vitro data, which have been developed extensively in Listeria since Listeria is a favored organism for both microbiologists and immunologists alike.

This is a brief overview of the types of studies that have been done and is not intended to be an exhaustive review of Listeria virulence or immunological mechanisms associated with Listeria. But the focus is on what kind of data in these studies can be used to help us in development of models.

First, dealing with the issue of pathogen virulence. We might pose the question: Can experimental virulence studies be used to identify a range of relative Listeria virulence? If you'll look at our -- going back to our data sources, in looking at human studies, as we've heard, the outbreaks are focused on a small number of predominant serotypes: the 1/2a, 1/2b and 4b. Although, if you noticed in the slide on the outbreaks, the butter outbreak was mentioned in there. And I believe it was actually a serotype 3a. So, an exception to every rule, I guess.

And it's important here, I think, to remember when talking about these serotypes -- and also, the phagetypes and ribotypes -- that these data are essentially valuable epidemiologic tools but are not necessarily mechanistically related to the virulence of the organism as well, which I'm sure you're all aware of.

Next, please. One virulence factor that's been studied extensively in in-vitro studies is Listeriolysin O, which we've already heard discussed today. Essentially, it's produced by all clinical isolates of Listeria. And in-vitro studies have revealed that it's required for survival within macrophage cell lines, which are an important line of defense against Listeria. But this is also not an absolute in that the survival of even Listeriolysin O positive Listeria is actually limited in in-vitro studies to a small percentage of the bacteria, indicating that there is some selection or adaptation that goes on in this system, as well. Listeriolysin O negative strains, however, do not survive at all in these in-vitro macrophage survival models.

Functionally, the Listeriolysin O enables the organism to escape from the phagolysosome of the macrophage and mediate the next phase of its virulence cascade or mechanisms which would be the cell-to-cell spread. That is, Listeria can also invade nonphagocytic cells -- such as liver cells, for example, and move within epithelial cells -- and move within the cytoplasm and spread from cell to cell by means of actin polymerization. The molecule or the virulence determinate responsible for this is a surface protein Act A which mediates actin polymerization.

In addition to this, there are also a series of proteins involved in getting the organism into the cell in the first place. One of these is the Internalin protein InLA which facilitates adherence to and invasion of phagocytic cells.

Next, please. Looking at how these studies based on essentially salt culture models pan out in animal studies, it's observable that Listeriolysin strains are all -- Listeriolysin O negative strains are avirulent in mice in parenteral and oral inoculation studies.

In addition to this, Act A negative strains also show reduced infectivity in mice. And, finally, another group of virulence determinates, the phospholipases, play an important role in the ability of Listeria to evade the early host neutrophil-mediated defense mechanism in the mouse liver, which has been shown in in-vitro studies.

So, we can look at what some of this data tells us in terms of dose-response in the next slide. In this study, this is a study based on oral inoculation and shows a reduction in the number of colony-forming units in the mouse spleen and liver comparing hemolysin positive and hemolysin negative Listeria strains. So, this gives us a kind of quantitative data based on the presence or absence of hemolysin in an oral inoculation model.

The next example shows the fact -- basically, the take-home message from this is that the Listeriolycin is not the whole story in terms of in-vivo virulence in the animal models in that strains which have the Listeriolycin but lack the phospholipase C are reduced in virulence.

Putting all the sort of animal virulence factor studies together into a model of what happens in the oral infection in the mouse model in Listeria, you could summarize it by saying that Listeria can attach via the attachment virulence factors to either M-cells in the gut or gut epithelial cells, become internalized, then move through the cell via means of actin polymerization and emerge on the other side of the gut barrier to be taken up by macrophages, which they are capable of survival in, and from there they're capable of then disseminating to various tissues and causing various pathologies in the animal.

Next, please. Looking at the last component of the dose-response parameters, host susceptibility, the question that we're posing here is: Can animal models of immunocompromised states provide us with any useful quantitative data on relative susceptibility in humans? This is a fairly ambitious question. However, I think that as we progress through there, you can see that there may be some relationships that are possible to exploit in this question.

We know from looking at human studies that healthy adults are usually asymptomatic carriers. Nonperinatal disease usually occurs in individuals as various predisposing conditions. For example, pregnancy, very young, infants, individuals with AIDS -- although, this is actually kind of an interesting case because parenthetically, when the AIDS epidemic first developed, it was initially thought that Listeria would be a common opportunistic infection. And, in fact, it turned out to be actually a sort of unusual opportunistic infection in AIDS, relatively speaking. And there's a reason for that which will emerge later on in the discussion. Cancer, immunosuppressive therapies of various kinds and, finally, old age are other predisposing conditions.

What you can say about this is that all of these predisposing conditions are likely to involve different types of immunosuppression mechanisms. That is to say, the factors that predispose in pregnancy are probably different than the factors that may predispose in cancer or in infancy or in old age on a mechanistic level. And this is more or less what the mouse animal model of Listeria infection tells us.

In fact, one of the most useful of these models and instructive has been the use of the severe combined immunodeficiency mouse model. And it was this model that led to the realization that there was an extremely important interaction of innate and adaptive immune systems in the mouse. That is that the SCIDS mice, the immune-deficient mice which lack either both T-cells and

B-cells, do not clear an infection but also, at the same time, do not succumb readily to the infection. In fact, they remain chronically infected, which was kind of a surprise at the time of the initial observation, I would think.

The neutralization, however, of the Cytokine Interleukin 12 or tumor necrosis factor L for either one of those results in an increase in the lethality of the infection in SCIDS mice and an increase in CFUs to quantify it again, thinking always of what quantitative data we can get from this, by between 1 and 3 logs.

The take-home message from the SCIDS mouse model is that in the absence of T-cells, the infection is controlled but not eliminated. Various studies have demonstrated that this effect is mediated by the polymorphic nuclear leukocytes, neutrophils -- primarily, monocytes, which are producing Interleukin 12 -- and NK-cells, which are present in these animals which produce NK or natural killer cells, which produce gamma interferon, which is one of the most important host-resistance mediators in the mouse model of Listeria.

On the next slide, this model, the SCIDS model is summarized by showing on the top, "SCIDS Mice," which remain heavily infected, chronically infected with

Listeria. But the Listeria is held in check by the innate immune system mechanisms -- that is, the NK cells and the neutrophil populations.

In the normal mice, these things are operating early on in the infection until such time as the T-cell mediated mechanisms kick in, resulting eventually in sterile immunity in this model.

Looking at the next slide, you can see that this has a direct impact on the dose-response to Listeria in a system where neutrophils are depleted by a monoclonal antibody against the neutrophil determinant. The dose-response effect is really quite remarkable. That is, the infective -- the lethal dose in this system essentially drops from four times ten to the eighth to four times ten to the fourth or a four-log increase in susceptibility, you might put it, in this particular mouse model in that zero of five of these -- it may even go lower than this -- zero of five of the controls are killed, whereas three of five of the neutrophil-depleted animals are killed.

Next, please. The purpose of this slide is not to have you figure out one single thing that's on this. This is the pathway of the -- and I put it up here for the point of showing that extensive studies have been done to show, to elucidate the various pathways involved in resistance.

The point is that within these various mechanistic studies are embedded information on dose-response to Listeria that is linked specifically to certain kinds of immune mechanisms. These I have tried to summarize on the next slide. Looking at various types of ways to manipulate this system, you can see that recombinant Interleukin-1 administered to the mouse results in a 250-fold decrease in the level of infection in the spleen.

Looking at the Interleukin 6 knockout, there's a 300-fold effect. That is a knockout animal. But this animal lacks Interleukin-6; therefore, in the absence of that component of the immune system, there's a 300-fold increase in CFUs.

Using, again, a monoclonal antibody to deplete Interleukin-12, there's a 500-fold effect. Gamma interferon is a thousand-fold effect. TNF alpha, also a thousand-fold effect in the mouse model.

I wanted to also mention at this point, while we're on the topic of Cytokines, what is happening and some of the events that go on in the pregnancy model as well because they fit in nicely to what we know from the mouse studies. And that is that there's studies in both human and animal systems that show there's actually an inhibition of NK cell function during pregnancy. And we know from the animal studies that NK cells are extremely important in the resistance to Listeria infection.

In addition to this, there's a shifting of the T-cell responses during pregnancy towards what's called a Th2 or T-helper-2 type Cytokine secretion pattern. That is, Interleukin-1, Interleukin-5 and Interleukin-10 are produced. It's also been shown in other -- in studies in the mouse model that the inhibition of Interleukin-4 actually has a beneficial effect on survival of mice infected with Listeria monocytogenes so that those things which tend to favor production of IL-2 are actually detrimental in -- of Interleukin-4 are actually detrimental in terms of the infection. And this is one of the events that's going on during pregnancy.

In addition to this, it's also been reported that spontaneous abortions in humans are associated with an increase in the sort of yin-to-the-yang here, the Th1 Cytokine. When this type of response gains predominance, it essentially begins to recognize the fetus as a foreign body and reject it. And it's worth noting that Listeria is one of the prime ways to attempt to drive this kind of response. So, there may be a link there in the human system that's doing what we can see in the animals.

Finally, of course, in terms of these animal studies, there are some serious questions that need to be asked about the use of these various animal models. First of all, would be: Does the use of gene knockout or monoclonal antibody-based deletion have any relevance in humans?

Secondly, do the mechanisms defined in the mouse model operate in human infections? There's very, very little information on what is happening mechanistically in human Listeriosis, at least that I've found. Maybe, again, some of the committee members know more information that I'm not aware of.

And finally, a kind of subset to this: Can the host-resistance mechanisms identified in the animal studies be connected with human biomarkers of exposure and susceptibility? That is, can we use what we know are important biomarkers in animals -- gamma interferon, TNF alpha, for example -- and use them to answer questions about human exposure and susceptibility to Listeria?

In the next slide, this is kind of a bit of a tongue-in-cheek slide in a sense, coming from the Washington Post just this past May 13th. Not to give anyone the impression that the Centers and FDA might be working at cross-purposes in some instances. But the recently-approved drug, Enbrel, which has produced spectacular results in treatment of rheumatoid arthritis, may have caused serious infections in some patients, six of who have died.

Enbrel is a biological response modifier, chemically engineered to attract and neutralize tumor necrosis factor alpha. Therefore, there is some relationship in terms of what we know, at least about Listeria infection in mice and these kinds of drugs.

In addition to that, one could only anticipate that as more of these mechanisms are investigated and the drug design becomes more sophisticated, there will be more and more therapies like this that are not just general immunosuppressive therapies, but very specifically targeted to certain immune mechanisms. So that there may be more and more instances where sort of designer drugs can knock out specific components of the immune system to a good effect in the treatment of inflammatory disease, but to a possible detrimental effect in terms of susceptibility to illness.

Secondly, as has been mentioned previously, we're in the process of developing in conjunction with the University of Georgia the Rhesus-pregnancy model. And in addition to the dose-response data -- which will undoubtedly not be available for the July 6th deadline, but hopefully sometime in the future, the absolute numbers in dose-response -- we're also trying to develop some biomarker data in conjunction with that study so that we can then if not look at -- if we can then verify what's happening in the mouse model and this sort of closely-related non-human primate model, it may go a long way to validating the use of the animal data in terms of modelling the relative susceptibility.

Next, please. Going back to the first slide and sort of summing it up and restating or stating maybe clearly for the first time, how we're going to use these various pieces of data or how we're proposing to use these pieces of data, in terms of the issue of numbers of organisms and food matrix, we're proposing to develop distributions for probability of illness based on the human data.

Ultimately, we hope in the future to be able to incorporate information from the dose-response studies ongoing now when they become available. We also will, hopefully, as more information from epi-studies comes available, that will also be incorporated. But at the present, we're essentially operating from the same data set that other risk assessment efforts have operated from in terms of human data.

Next, please. In terms of organism virulence, we're proposing the concept of using the in-vitro and animal data to model a range of virulence for Listeria monocytogenes to determine -- rather than a sort of a plus-minus virulence situation, to see if that could be-- help refine the model.

And, finally, in terms of host susceptibility, we're hoping to explore the use of the animal, primarily mouse data, to model relative susceptibility in various immune-compromised states. Ultimately, we would like to correlate the mouse biomarkers with the primate model as surrogates for human infection.

And that's pretty much the status of the dose-response effort and data forces. Thank you.

MR. MICHAEL JAHNCKE: Thank you, Dr. Raybourne, for an excellent presentation.

We're going to just break from regular procedure a little bit. It's warm in this room, and our audience is probably wilting. We're going to take a 20-minute break. What that will allow people to do is to break down this wall and open up the two rooms to air this out a little bit. And then the next one will be our committee discussion with all the speakers and our National Advisory people.

So, 2:25, come on back.

(Whereupon, a recess was had in this matter.

MR. MICHAEL JAHNCKE: Let us get started on the afternoon session. Before we do, there's one little housekeeping point. Committee members need to turn in their -- they've got a calendar for August through December as far as availability for meetings. Fill that out and leave it with the staff in the hallway.

We're going to have our committee discussion with all the committee members plus the presenters for today. Keep in mind, if there are any questions that any of you have about the document itself, now is the time to bring those up. And also, keep in mind the three questions that were first presented this morning.

Question one: Is the scientific approach sound? The second question was: Do they have all the right data? And the third one is: Have they overlooked anything? With that, we'll -- Yes?

DR. ALISON O'BRIEN: I'm not a regular member of this committee. May I ask a question?

MR. MICHAEL JAHNCKE: Absolutely. Identify yourself.

DR. ALISON O'BRIEN: I'm a member of the Food Safety Committee. It's Dr. Alison O'Brien.

I wanted to ask a question of the last speaker, Dr. Raybourne, who is right next to me. Dr. Raybourne, you were talking about using animal model data, pili mouse model data as part of your dose-response assessment, guesstimates, estimates.

Are you aware of the older data from Christina Cheers (phonetic) looking at innate susceptibility of different mouse strains to Listeria? Because there was nothing about the basic genetic host background in your discussions today. You talked about Cytokine response being modulated. And I can't remember, unfortunately -- I'm gonna say what I think she found. And she found there was a gene in mice that controlled early response to infection which allowed certain strains of mice to be several logs more susceptible to Listeria than others. I think it was a complement, actually, complement-medicated factor on mouse chromosome 5. And you never -- I could be wrong about that, and I don't want to mislead. But there's a whole set of data on that.

DR. RICHARD RAYBOURNE: Rich Raybourne, FDA.

Yes, I'm aware of that data. I think it's kind of, as I recall, almost a mirror image of the salmonella ITY data; is it not?

DR. ALISON O'BRIEN: It is not.

DR. RICHARD RAYBOURNE: The strains are different, though.

DR. ALISON O'BRIEN: It is not the same gene; and it doesn't have exactly the same mouse profile in the product, no. But the product of the gene is not ITY, IEN RAM now. It's a different gene, and I think it affected complement, C-5 component of complement. I believe the AJ strain of mice, which is low in that complement component, was particularly susceptible to Listeria.

So, since you're using mouse models, I thought you might go back and check that. My data may be wrong, but I know it isn't the same profile as salmonella exactly.

DR. RICHARD RAYBOURNE: Yeah, that's -- I'm agreeing with you. I'm saying it's not the same.

DR. ALISON O'BRIEN: Oh, it's not the same.

DR. RICHARD RAYBOURNE: I think it's -- in the C-57 is relatively more resistant in Listeria and it's more susceptible in salmonella.

MR. MICHAEL JAHNCKE: Other questions?

DR. RICHARD RAYBOURNE: But that's a good point. Thank you.

MR. MICHAEL JAHNCKE: Bruce?

MR. BRUCE TOMPKIN: Bruce Tompkin. I just had one question. Both of you mentioned carriage, asymptomatic carriage. Another one was the phrase where healthy adults are usually asymptomatic carriers. Is this a reality? Are there carriers whereby normal, healthy individuals may have an indigenous population of Listeria monocytogenes in the GI tract? Or is it a transient, just as a result of consuming food; and when stool surveys are conducted, they merely show up as a positive because of whatever exposure?

DR. PATRICK McCARTHY: In the studies that I referred to, they were point prevalence. And so, they simply were there at the time. In the German studies, several thousand people were involved. And they found it in those individuals.

They found higher rates when they tested the same person over a period of time. It's my understanding that there are people that are carrying the organism but do not show symptoms. How long they carry the organism, I don't know.

MR. MICHAEL JAHNCKE: Yes, David?

MR. DAVID ACHESON: David Acheson. That, to me, raises of the question of any data out there on person-to-person transmission.

DR. PATRICK McCARTHY: This is Pat McCarthy: I did see one study -- and, of course, I can't remember exactly the name of the study at this time -- but there was a suggestion that individuals living in the same household may have -- there may have been transmission person-to-person. But, for the most part, in all the studies I looked at, that was not an issue. Person-to-person transmission was not an issue.

MR. MICHAEL JAHNCKE: Other questions from the committee? Yes, Michael?

MR. MICHAEL DOYLE: This is Mike Doyle. Richard, I think I noticed on your slide, you had a estimated dose of ten to the ninth for the butter-associated outbreak. Did I read that right?

DR. RICHARD RAYBOURNE: Rich Raybourne. It should not -- if that's what it said, it shouldn't have said that. I think the dose was, as I recall, ranging between a hundred and about ten to the fourth.

MR. MICHAEL DOYLE: Yeah. That was the count from the butter. But above that, I think I saw ten to the ninth. And I was curious to know how you arrived at that number.

DR. RICHARD RAYBOURNE: No. I think the number is much lower than that.

MR. MICHAEL JAHNCKE: Other questions? Bruce?

MR. BRUCE TOMPKIN: We haven't really discussed the document. And I only have two questions. The simplest is figure one. I've tried to understand it, and I don't. And there's no sense spending time on it now. But I couldn't figure it out -- the top portion, in particular.

But my other comment really was relating to Page 6. And as part of the background information where this is just all background and introduction, Pages 4, 5 and 6, and it's not in here -- and I'd just like to suggest perhaps you may wish to do this -- but it is to actually compare the policies. I know the intent of this risk assessment is not to address policy at this point in time. But as a matter of comparison, I thought it would be helpful to compare the policies in other comparable countries, industrialized countries, in terms of their Listeria policies, the numbers of cases per hundred thousand -- and I know CDC will wince at that thought because no one has as good a system as the United States what the data are saying.

Anyway, the number of cases per hundred thousand and also any information on percent of positive food samples with the intent to see whether or not there's any relationship between the policy, the actual exposure in terms of percent positive foods that are reported in those countries, and then the public health impact. And that would just be a matter of background information at this point. That's all it would be. It would not be anything actionable, as I understand, from this risk assessment.

MR. MICHAEL JAHNCKE: Cathy?

MS. CATHY DONNELLY: Cathy Donnelly. I'd just like to follow up on Bruce's point and put in a plug for a comment that was made earlier today in the public comments section. And that being a focus on production practices that leads to production of a food. And the case that was being discussed was cheeses, and the focus of the risk assessment was on food type or cheese type. And I'd like to put in an appeal for production practices, i.e. farmstead cheese versus cheeses made in the manufacturing plant. And I think you'll see a big difference in incidents.

MR. MICHAEL JAHNCKE: Other comments, questions? Yes, Dane?

MR. DANE BERNARD: Thank you. Dane Bernard, not an immunologist. So, take your question for what it's worth. The fine report on how we're gonna model immune response, how do you plan to take what you've got and translate that into what I think most of us would accept as a population who distributes a wide range of immunological conditions which vary. I guess I'm just curious because we've got models that show different parts of how the immune response can be activated or not activated against this particular challenge.

But how do you go from where we're at now to what you, what I think will need to do, which is look at the human condition and the whole host of immunological conditions from whatever you call normal or whatever we rank as normal down to those who are very, very severely immunocompromised?

DR. RICHARD RAYBOURNE: Rich Raybourne, FDA. I think that the issue that you're raising is one that we're at the moment struggling with as well. I think that clearly there's a spectrum of -- going to be a spectrum of immunocompromised individuals. I don't think at the moment we have a good handle on ways that we can realistically measure that in the population as a whole to even get it, to get at what proportion of the population is, quote, unquote, "immunocompromised" and to what degree they're immunocompromised. It's kind of a technically daunting task.

I think that the positive side of using the data that I -- of sort -- of the type that I presented is that it's at least a quantifiable measure as opposed to kind of a rough approximation. I think we need to try to also in as many ways as we can make sure that what we learn from the animal models, particularly the mouse models, is translatable into the human situation. This is particularly difficult in Listeria because there's essentially no prospect for doing any kind of human clinical trials in Listeriosis. And so, the best approach that we have right now is to try to develop a surrogate model, which we're trying to do in a primate, in a primate system.

It might also be possible, for example, to develop some of this kind of correlative human data in outbreaks or in following up on patients involved in outbreaks. But it just hasn't really been done to any extent at the present time. So, it's a good issue, but I'm sorry we don't have a better answer at the moment for you.

MR. DANE BERNARD: Follow-up, if I might?

We've got data on those populations which seem to be more at-risk -- this is outbreak data -- who gets Listeriosis predominantly and who doesn't. We know enough, I think-- not an immunologist. We know enough basis, what you've presented, I think, to theorize what some of the mechanisms of susceptibility might be in those categories.

Have you thought into that scenario to see if there's any mileage there? I mean, for example, the less than one-year-old group. We know the immune system is still developing, immature, unchallenged, da, da, da, da. Based on the mouse models that you've got, is there anything that applies there? At the other end of the spectrum, same thing.

DR. RICHARD RAYBOURNE: Rich Raybourne again. I think in terms of doing those kinds of studies, we should look at, for example, in levels of quantifiable types of markers, like the Cytokines I mentioned, in these populous -- it's theoretically possible to do that. The problem with doing that -- at least my understanding of it -- is in the absence of an ongoing infection measuring levels of circulating Cytokines is not going to be very worthwhile. And at the very least, what you would want to do to get into sort of a more technical way of approaching this, if I could, what you would want to do is to somehow collect materials from these individuals, stimulate them in-vitro and look at the ability of the cells to respond. I mean, it would be a huge and expensive task to do this kind of thing.

There may be other simpler ways you can measure this, looking at -- and non-invasive ways, too. And we're currently trying to think of approaches to this in terms of even to the point of doing serological-type surveys, although this is problematic in Listeria because there's not a lot of evidence that I'm aware of that serum antibody responses are important in resistance to Listeria. So, I mean, it's a great question. I wish we could answer it and come up with an approach to it. And we've certainly thought about it but have not done that at this point.

MR. MORRIS POTTER: Morris Potter. Rich, I think what Dane is suggesting is that say, for instance, in the geriatric literature, it's known more or less which subsets go first. And, therefore, if we can look at susceptibilities of various mouse strains that are absent, those things that go in 50-year-olds and then the things that start to go when we hit 60 and so forth, that we might be able to then model the human population for those age groups and suggest when people are going to become more susceptible to infection, when they're going to become more susceptible to serious invasive disease and that sort of thing.

MR. WILLIAM JAHNCKE: Bob?

MR. ROBERT BUCHANAN: Bob Buchanan, FDA. Yeah, I think I'd like to echo on what Morrie says. I'm wondering if you may be making this more complicated than is warranted considering the huge range of -- and certainly, you're going to face with the rest of your risk assessment. Morrie and Jim Smith and I did a presentation a bunch of years ago on trying to get some estimates of increased risks associated with aging. And while certainly you're gonna have to come up with some kind of fudge factor to relate the increased susceptibility, it was not very difficult to find some age-related decreases in, for example, T-cell proliferation. It was not difficult to come up with age-related equations that we could develop for achlorhydria in the aged. So, I'm wondering if we couldn't just start off with trying a couple of fairly simple relationships that have been gleaned from these broad population studies, start simple. And if it didn't work, then get more sophisticated.

DR. RICHARD RAYBOURNE: Rich Raybourne again. I think that's a good approach, yes.

MR. MICHAEL JAHNCKE: Yes?

DR. WESLEY LONG: I do want to make one point, though.

MR. MICHAEL JAHNCKE: Identify yourself, please, Wes.

DR. WESLEY LONG: Wes Long, FDA. That it's consistent with some of our conversations yesterday that what we're doing is, you know, we don't have all the data now certainly, clearly. But what we're doing is laying a framework at this stage and using that to figure out what to do next. And we talked about how we can modify the risk assessments as more information becomes available. So, I think this sort of thinking is important.

Rich sort of mixed up the data we'd like to get from outbreaks, that sort of thing, which is future, which we don't have now. But by doing this sort of thinking now, I'm hoping that we will sort of lay the groundwork, even though we may not be able to utilize some of the things that he's talking about immediately.

MR. MICHAEL JAHNCKE: Yes?

DR. ALISON O'BRIEN: This is Alison O'Brien. Following up on what Bob Buchanan said about T-cell proliferation, some kind of marker that suggests you might be more susceptible to Listeria. The question goes back to something Dr. Raybourne said during his talk. Why aren't a lot of AIDS patients infected with Listeria, or are there? I mean, I know that I saw that as a subcategory. But to me, it seems a surprisingly small portion, given that if we accept the paradigm that this is an organism that uses protective immunity as related to cell-mediated immunity, not pneumo-immunity.

DR. RICHARD RAYBOURNE: Rich Raybourne again. In terms of the AIDS question, I think part of the answer -- and you're right. It's not as common as you would think it would be among AIDS patients. And this was one of the sort of statements in their first papers that came out when there were finally some Listeria AIDS cases. And I think part of the reason for that may relate to the observations with the effects of, for example, the Interleukin 4 and the fact that it acts -- which in CD-4 deficient patients, is going to be lower. And in the mouse model, when you neutralize -- and this is not a complete answer, but it's sort of a clue -- that if you neutralize IL-4, you actually ameliorate the Listeria infection in the mouse model. So, it has kind of a detrimental effect.

MR. MICHAEL JAHNCKE: Yes, go ahead.

DR. PATRICK McCARTHY: This is Pat McCarthy. It's true that in AIDS patients, in some of the earlier literature, researchers were saying that it's not very common in the AIDS patients. But then about '86, '87, '88, there was an estimate that AIDS patients have Listeriosis about 150 times more often. Then, more recent, I believe there was another estimate that AIDS patients may have Listeriosis about 280 times as often.

So, it's true that in the beginning, the researchers were wondering why Listeriosis wasn't showing up in AIDS patients. But as more information became available, estimates started to increase.

MR. MICHAEL JAHNCKE: Yes, Bob?

MR. ROBERT BUCHANAN: Bob Buchanan, FDA. Yeah, I just wanted to affirm that. My recollection was that the approximate increase in risk associated with Listeriosis and AIDS was about 300-fold. So, I think there is a very substantial increase in risk.

MR. MICHAEL JAHNCKE: Dane?

MR. DANE BERNARD: Thank you. Dane Bernard, not an immunologist. Still not. But we're not on immunology.

Another factor, I think, when you look at the data on incidence of Listeriosis in people with AIDS, you've got to look at the interventions that go on there as well. Prophylactic use of antibiotics, extensive dietary advice, is all provided once a person is diagnosed in that category. So, there's a risk mitigation or risk management strategy there, I think, that has a strong intervention that you're seeing showing up in health statistics.

MR. MICHAEL JAHNCKE: Other comments and questions from the committee?

Yes, Bruce?

MR. BRUCE TOMPKIN: This is Bruce Tompkin. I'd like to have a little clearer understanding as to how information is to be given to the risk assessment team. If someone has data, do they just say, "Here, Dick Whiting. Here it is"? Or is there a mechanism -- I know that you went through with a published announcement in the Federal Register, and so there's probably a formal mechanism. But how do we know that information provided will be used or considered and so on? And once provided, to what degree -- I know this process is one of the processes we're going through now. This is a public process. This is an open process. So, I assume data that's provided will become public or available to the public.

Could you help me with that a little bit?

DR. RICHARD WHITING: Richard Whiting. Yeah. There's a paragraph or two in that Federal Register Notice as a result of some of the discussions with us and our lawyers and so on, that I think we're probably breaking some new ground for FDA here, as well.

The information that would be submitted, I think you would have to expect that it would become public information. But we did say in there that we would accept information that has been summarized or blinded and various terminologies like this. So, if, say, the meat industry, for example, through one of your trade associations wanted to do a quick survey of whatever Listeria your members might have, and the trade association would just present a summary to us, that would be the information that we would have.

And as risk assessors, we would then try to evaluate that information as best we can. The more information, the more details you could provide, the more useful the information would be to us. But we'll accept what is offered. I would like to think that if some data came in, we might have an indication of what methods were used, what sensitivity -- if it was presence/absence data, what sensitivity might be there.

But, again, we will just accept to try to use whatever people are willing to submit to us. And we recognize this is sort of a new situation, I think, for all of us. And we're gonna try to use this as a scientific process and not a regulatory process, and I guess we'll have to see how it goes.

MR. MORRIS POTTER: This is Morris Potter. If I could amplify on that a little bit. Part of the rationale for using risk assessment is that it's a transparent process and that people who look at the risk assessment ought to be able to repeat it using different assumptions. And that does create a need to make the data sources available. If there are data that could be useful for the risk assessment but that might be felt inappropriate to become public, they may still be useful in terms of trying to validate things internally. But I think that our preference is to use risk assessment to help in making our decisions on risk more transparent, more understandable to the broader audience.

We wouldn't want to turn our backs on data that could be useful. And if you have things that you'd like to discuss, we can chat with you.

Wes, did you have any clarification on that?

DR. WESLEY LONG: I do. First of all -- This is Wes Long, FDA. I have the answer to this because it was reported in Food Chemical News on the back cover page there about six weeks ago that the Risk Assessment Clearing House set up through the Food Safety Initiative was going to be collecting the Listeria data.

I'm not sure what the source of that information was. But there is a Risk Assessment Clearinghouse that is at the University of Maryland that's a part of FDA's new Joint Institute for Food Safety and Applied Nutrition. And we are in the process of -- This clearinghouse is intended to be a repository for data methods, models, anything to do with risk assessment, initially focusing on microbial risk assessment needs.

Dave Lineback (phonetic), who is the -- I'm not sure of his title, the chair or the head --

MR. MICHAEL JAHNCKE: Director.

DR. WESLEY LONG: The Director, thank you. The Director of the Joint Institute for Food Safety and Applied Nutrition will take responsibility -- at this point, we're not really set up, but we could be set up very soon to take data. He will take the responsibility to do sort of a secondary cleansing of data. I think you would want to -- if you had to blind the data, you would probably want to do that first. But he would be a second mechanism to do that. And he would provide the assurance, again, that -- of course, all of the information that goes in the Clearinghouse does become public. But that FDA would never -- this is an opportunity to blind the data again and further assure submitters of the confidentiality of that information.

So, Dave Lineback -- I could give you information about how to get in contact with him. That might be another way to get that information.

MR. MICHAEL JAHNCKE: Morrie?

MR. MORRIS POTTER: I guess if I could recap where we are, Richard has suggested that there is information in the Federal Register where you can send it. Wes has suggested that you could send it to Dave. And, in fact, you could also send it to Richard or to me or to Joe Levitt (phonetic) or to anybody else in the building, and we will see that it gets to the right place.

MR. MICHAEL JAHNCKE: Yes, Bob?

MR. ROBERT BUCHANAN: And to answer the second half of your question, Bruce, on how to tell whether or not the data is being used similar to the document that you have in front of you that outlines the data sources that are being considered, the risk assessment itself will detail the data that was selected for use and the criteria for using it. So, if your data didn't get used, you would know it by reading the final document.

MR. MICHAEL JAHNCKE: Other comments and questions? Yes, Dane?

MR. DANE BERNARD: Thank you. Dane Bernard. Pat, you covered a number of things in your review of the epidemiological information. Some of us, I think, who have watched outbreak information and epidemiological studies for some years, occasionally run across things that we don't necessarily agree with, that maybe they weren't in fact quite as well-established as we thought they might have been.

Is there any need to or will you be reviewing any of the source information on past studies to see whether it meets some kind of criteria of acceptability in terms of whether we, in fact, have targeted all the right foods or maybe have targeted one or two too many as being implicated in outbreaks?

MR. MICHAEL JAHNCKE: Please identify yourself.

DR. PATRICK McCARTHY: Pat McCarthy. Before I refer some data over to Clark Carrington who's going to be doing the modelling, I am going to look at it to make sure that it seems reasonable to me that the cases are well-described and that it has the basic information in there, including the rationale for implicating the particular food.

I'm going to try to summarize the data a little bit, in addition to giving them the raw data. But summarize the data a little bit to give them an indication of how often a particular food or type of food is being referred to in the studies that I refer to. So, yes, I'm going to try to be critical in terms of which studies are referred.

MR. MICHAEL JAHNCKE: Bob?

MR. ROBERT BUCHANAN: Bob Buchanan, FDA. In yesterday's session on vibrio parahaemolyticus, we spent quite a long time on the dose-response area talking about multiple biological end points and what would be appropriate to model in the case of vibrio. And that's a fairly classic enteric pathogen. You deal with colonization of the intestinal tract as one biological end point.

Sepsis is a second biological end point. Gastroenteritis is an intermediate biological end point. In your presentation, Pat, you gave several different potential biological end points. And, Rich, you provided a model for infection that would not be too dissimilar from what we were discussing yesterday on vibrio.

Have you decided yet on what will be your biological end points that you're going to be modelling or considering? Are you going to do multiple ones? Is it going to be one for sepsis, one for meningitis, one for neonates?

DR. PATRICK McCARTHY: This is Pat McCarthy. I had planned to take a look at the studies and, again, to put them together in terms of -- in a lot of different ways. Organize the data for the model in a lot of different ways. And, certainly, sepsis and meningitis are two big end points. I was going to try to give the modeler an estimate of how often those particular end points come up in the literature that I reviewed. And also, since the more mild symptoms, there seems to be several studies that refer to mild symptoms, I was also going to give them an estimate of how often that seems to show up.

In terms of headache or chills or abdominal pain, I'm not there yet in terms of how I'm going to group that data; but it might be -- I do have estimates in different papers of how often subjects or cases had diarrhea or had chills. And so, I haven't really decided how I'm going to give it to them, but I'm going to try to be open when I do and give it to him the way that's most productive for him.

MR. MICHAEL JAHNCKE: Yes, Bob?

MR. ROBERT BUCHANAN: Sort of a follow-up on this, now directed towards Rich.

Rich, do we have any estimates on the probability of colonization or attachment? In presenting your model of the infection, you just sort of said "attachment," and you didn't really deal with that.

Do we have any estimates on what it takes to get attachment, or are there different known attachment mechanisms? Can you come up with any kind of probability of attachment?

MR. MICHAEL JAHNCKE: Identify yourself, please.

DR. RICHARD RAYBOURNE: Yes. Rich Raybourne, FDA.

In the model I presented, I mentioned the Internalin, which is essentially an attachment-type virulence determinant. In terms of numbers associated with colonization, I'm not aware -- I don't have that information right now. There may, in fact, be some because a number of oral infectivity studies have been done. And whether they used attachment as an end point or not, I'm aware of one study where they looked at invasion in the intestinal wall and quantified organisms invading the intestine of the mouse. But beyond that, no, I don't know of any.

MR. MICHAEL JAHNCKE: Other questions and comments?

Our next presenter, before we have it, on behalf of the subcommittee, we'd certainly like to express our appreciation to all the presenters today and all the hard work. The product is coming along quite nicely. Thank you very much.

Our next presenter is Dr. Richard Whiting. And he is going to be giving a summary of what has been presented and discussed, presented today.

DR. RICHARD WHITING: I'm just going to be very brief in light of this good discussion we had. But maybe we can start with Bruce's question on this disputed figure here. This is the one Bruce is referring to. We did have some comments on the draft phase, but I guess we didn't get around to revising it. But it's just trying to say here at the top, "Food consumption, food contamination." These two go together to form your exposure. And then this middle part, "Food Vehicle Virulence and Susceptibility" is the disease triangle idea, the hazard assessment, and that leading on to illness. It's trying to give a little sense of flow to it. I guess I can see we can do a little bit of editing and reworking of that to make it a little bit more clear.

There's kind of an old saying that risk assessment people have had, "Let the data speak." And I guess that's largely where we are at this point in the risk assessment. You can see we've accumulated a lot of information. We've given you some ideas of where we would like to go with it.

The next stage for the risk assessors is to try to take all of this information and, really, just see what we can do with it, see what the information can be summarized as. And I think you've seen the problems with trying to combine the information on the presence of Listeria in foods with the consumption.

That data base that Mary has -- I forgot. She had something like eight or ten different categories of hamburgers. And then when you get to data like the cheeses, we have some good consumption information on some of the Hispanic cheeses, for example. But then these data bases don't say anything about whether this was pasteurized or unpasteurized cheese. And this is the kind of information that we have to try now to pool together and bring out of it the conclusions that we feel are justified in bringing out. And we may find there will be quite a few areas where we will just say there is not information available that we can go further. And part of the exercise of doing a risk assessment like this is to highlight the data gaps.

I also think that this will be an iterative-type of process. I think it will be occurring both within the next few months, and I can see us doing an initial summary of the data, which will perhaps highlight certain areas that we will then go back out and try to find more detailed information on.

And I can see sort of this second round as a point where we will probably try to get in contact with various people in the industry who might have information on specific consumptions, you know, my question of pasteurized versus unpasteurized in certain groups of cheeses. Or perhaps the industry might have some information on consumption patterns or food preparation habits. Various questions like this which we don't have yet but might be very relevant for particular classes of food.

So, I see this as an iterative-type process, and it will probably continue beyond the September, October date that we have set as a target for completion of the first part of the risk assessment.

And we've also made quite a few references here today to various research projects that are underway, the primate pregnant monkey primate study, and various studies like this -- which obviously won't be ready by September and October but yet, obviously, we want to take that and look at the risk assessment again as soon as that data becomes available.

I have been quite heartened today by, I think, the sense of participation here by the industry. I was on the Salmonella enteritis and egg risk assessment team that the USDA did a year or so ago. And at that point, we sort of approached the industry. And I would say they approached us back with quite a bit of trepidation. And we really did not get very much back that was helpful to it. I think there was a lot of apprehension about what the whole risk assessment process is about. I hope everyone is becoming a little bit more aware of just what a risk assessment is and what it does and that people will be a little more willing to participate in this. I think for all of us, our goals are increased food safety.

And I really do put a plug out there and echo again the conversation we did just have about the submission of data and blinded data. And I really do put an invitation out to industry and everyone to become active and follow it. And if you have specific information that you can bring to us, that you do that.

So, with that, Mr. Chairman, I thank you very much.

MR. MICHAEL JAHNCKE: Thank you very much. Again, thanks to your entire group. Certainly appreciate it and thank you.

Morrie, I'll turn this over to you.

MR. MORRIS POTTER: I'd like to add congratulations to the risk assessment team. That was very nicely done. And on behalf of FDA and FSIS, I would like once more to invite participants today who are not members of the committee to come to the mike, identify yourself and make whatever statements you'd like to about the risk assessment model that was presented today, the direction the team is taking or other comments on the risk of foodborne Listeriosis.

Perhaps while people are thinking about that, I'd like to direct a question to Wally. The question arose earlier about colonization. And I wondered if you had any information on either transient or long-term colonization from your clinical experience.

MR. WALLY SCHLECH: Thanks, Morrie. Wally Schlech from Delhausen. I don't have any information other than to say we did some carriage studies during the now-ancient maritime outbreak in family members, primarily, and did find some carriage. We assume that's probably because they were eating the same items in the menu and during the time we were sampling which, in fact, was often 30 -- probably several months after the case, were able to find some Listeria. But whether these were new items or leftover from the previous, I don't know.

I think there are some Dutch studies -- I believe in Europe, some old studies of longitudinal carriage, as I recall. I think, although most people would suggest that carriage is transient, that it may remain in the bowel flora for a period of time. So, I'm not certain.

I think I wanted to raise a question about the biological endpoints. I think the febrile gastroenteritis syndrome is very much a distinct entity. And the thing that wasn't talked about today is the extraordinarily high attack rates within the exposed group for that particular syndrome, whereas mostly the

Listeriosis you see, even in the outbreak situation, the attack rates in the overall population are quite low.

I think there is some evidence that that may be more related to a huge dose and possibly even local. So, maybe the hemolysin acts as a local cytotoxin in the gut for a period or something. I don't know.

But I think there is some data. I've done some work in gastrointestinal carriage in mice and rats. And we can see carriage persist for a couple of weeks in the droppings. But we haven't really gone beyond that at this point in time.

And there certainly doesn't seem to be any specific attachment factors. The Internalin protein, I think, is an interesting protein. But in terms of the things we think about, pili and other sort of typical attachment factors, Listeria doesn't exhibit them. And we really don't have any information there.

MR. MORRIS POTTER: Thanks, Wally. Other comments? In that case, I think we can wrap this up. Tomorrow morning we start again at 8:00 for the plenary session of the National Food Advisory Committee.

(Whereupon, the hearing in this matter was concluded at 3:20 p.m.)

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I, ANNE I. MAZIORKA, CERT, a Notary Public within and for the County of Cook and State of Illinois do hereby certify:

That the foregoing transcript was reported to me by electronic recording, was thereafter reduced to typewriting under my personal direction and constitutes a true record of the testimony given;

That the said hearing was taken before me at the time and place specified;

That the hearing was adjourned as stated herein;

That I am not a relative or employee or attorney or counsel, not a relative or employee of such attorney or counsel for any of the parties hereto, not interested directly or indirectly in the outcome of this action.

IN WITNESS WHEREOF, I do hereunto set my hand and affix my seal of office at Chicago, Illinois, this 11th day of June, 1999.

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ANNE I. MAZIORKA
Notary Public, State of Illinois
My Commission Expires 2/21/2000

Anne I. Maziorka
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